IMPACT OF FOOD ADDITIVES ON PROBIOTIC CULTURES

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By

RIMJHIM

Under the Supervision of:

Dr. Seema Kashyap

Department of Home Science

Co-Supervisor

Dr. Prem Saran T.

Department of Botany

DEPARTMENT OF HOME SCIENCE, FACULTY OF ARTS

DAYALBAGH EDUCATIONAL INSTITUTE, (DEEMED UNIVERSITY)

DAYALBAGH, AGRA (U.P) - 282005

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1. INTRODUCTION

In the recent years, globalization has affected every aspect of our lives. Globalization, by definition, is the process of sharing homogenous and intelligible culture. It can also be defined as reduction in barriers to trade, the cross-border movement of goods, services and capital resulting in an increased flow of commodities, which includes food produce. Enrichment and improvement in the quality of life with social harmony, peace and universal growth is a positive progress that has been made as a result of globalization, however emergent public concerns, in particular to the health and well-being of individuals has also gained relevance in context.

Globalization has had major impact on the food systems across the nations, as a result, food systems have been evolving and continues to do so, resulting in diversity and also increased availability due to evolved processing technologies that have rendered the lives of food, across the shelves, longer than what had been envisaged, and their nutritional composition more wholesome and composite than what it has ever been. Owing to increasing trends in global trade of food and food produce, the local markets, both urban and rural, are flooded with processed food. This in turn has further accelerated more of food processing and mass production of food that is designed, developed and delivered to the consumer's sensory perception and need. Needless to mention, the increased usage of food additives, that supposedly adds value to food, in terms of their sensory qualities (viz., Taste, texture, Aroma, Appearance and flavor), besides aiding them with a longer shelf life. Inclusion of food additives, namely, colors, stabilizers, thickeners, antioxidants, bleaching agents and preservatives has been a practice since the beginning of the era of industrialization and has become vital to the food industry today. While inclusion of these additives has been in practice to enhance the product appeal of a prospective consumer, vivid and stringent guidelines and regulations have been laid down by statutory bodies and authorities to govern and monitor their usage.

The implications of excessive usage of food additives and their health impact has been well established, however the impact of these additives on the natural flora of the food, in particular, the probiotic species, is a subject that still remains elusive. Probiotic bacterial species have known to be denizens of the gut microbial flora. Recent studies have also revealed that, dysbiosis (also called as dysbacteriosis or microbial imbalance or an impaired microbiota) of gut flora can cause diseases

(Swidsinski*et al*., 2008), therefore, healthy gut flora has been observed to be key to human health (Zhang *et al.*, 2014). Evidently, understanding the relevance of probiotic species in fermented foods, that adds to the gut flora has gained considerable importance in the recent times (Zhang *et al.,* 2017). These probiotics are a component of the fermented foods that is a major and important part of human diet in many parts of the world. Their importance and relevance in a diet and their growing awareness has put these processed probiotic foods on the shelves of almost every store and have become a common sight in the market. Although consumption of probiotic foods has its heritage dating back to century old food traditions and systems, the growing consumer demand for these foods is a trend that has been witnessed in the current decade. Yielding to the demand of the health-conscious consumers, manufacturers have made a natural plunge into mass production of the probiotic foods, which in the years to come is likely to grow multifold. As has been the case with other processed ready-to-consume food products, in order to increase the product appeal, use of food additives, namely, colouring agents, thickeners and stabilizers, that have been common among probiotics, are likely to increase.

Probiotics, as the name goes, are 'pro' to our health and wellbeing (biotic). These organisms have been studied to have a large and lasting impact on the physical and mental wellbeing on the consumers because of their role in release or in stimulating the release of neurotransmitters. Probiotic species of bacteria have been reported to affect the release/secretion or directly release or secrete neurotransmitters, namely Dopamine, Seretonin, Gamma- aminobutyric acid (GABA) etc.

The subject of study under consideration is to understand the effect of the food additives commonly used in probiotics and fermented products on the probiotic cultures, in terms of their release, secretion of neurotransmitters.

2. RATIONALE TO THE STUDY

Gut microbiota diversity is affected by so many factors and subsequently diet play major role in it and so as food additives that has vital functions in food but it is confirmed now from several researches that explored the effect of dietary habits play important role in damaging healthy gut microbiota diversity. In terms of adverse health effects of food ingredients such as food additive on gut microbiota is less explored. Several findings point out that food additive affect gut microbiota and have several heath disadvantages including physiological and psychological disorders. The role of

additives in gut brain axis and psychic functions is needed to study in the era of processed food products. This study will be conducted to clarify the link between food additives, probiotics and psychic functions through assessment of neurotransmitters released by probiotic species.

3. OBJECTIVES

i. To develop fermented milk products using different probiotic cultures.

ii. To study the effect of addition of permitted additives (Colours, Thickeners & Stabilizers) for fermented milk products on the growth of starter cultures, namely *Bifidiobacterium Spp.*, and *Bacillus Spp.*.

iii. To study the effect of addition of permitted additives (Colours, Thickeners & Stabilizers) for fermented milk products on the release of neurotransmitters by different probiotic cultures, namely *Bifidiobacterium Spp.*, and *Bacillus Spp*.

4. LITERATURE REVIEW

Food and Gut Microbiota

Human intestinal microbiota also referred to as microbiota or gut flora, are the microorganism that inhabit the digestive tract of human. The human body has over 1014 microorganism in the gastrointestinal tract or the gut, which is ten times higher than cells of human body itself (Backhed F., 2015 and Neish.,2009). In his narration, 10% Human (2015), AllanaCollen, while discussing the relationship between the human microbiota and health, throws light on the influence of the gut microbiota on the human wellbeing. Gut colonization by microbes begins right from birth and continues to persist, with modulation however, all through the lifespan of individuals (Tanaka et al., 2017). The modulation in the gut microbiota is obviously attributed to the diet of individuals and continues to be influenced by the changing diet patterns (Conlon et al., 2017).

On one hand as foods become the source or medium of carriage of the prospective gut microbial resident, on the other hand the composition of our diet in-particular the macronutrients (Carbohydrates, Lipids and Proteins) has been 7 reported to define the shape of our gut microbiota (Singh et al., 2017). The macronutrients act as the substrate supply to the already resident flora of the gut, which is to say that the composition of the diet, whether carbohydrate, protein or fat is likely to impact on the abundance and dominance of the microbial, mostly bacterial species amongst the

residing microbial flora of the gut. At the same time, as an eventuality of the microbial product derived from the breakdown of these macronutrient, the prevalence and sustenance of the category of the secondary population of species is defined (Conlon et al., 2017).

Reports have surfaced that impact of plant-based diet on the composition of the gut microbiota, wherein researchers discuss varying levels of Clostridium perferingens, Enterococcus spp., Lactobacillus spp., Eubacteriumrectale, species of Bacteriodes, Roseburia, Ruminococcus and Bifidiobacterium, upon consumption of broccoli, cereals (whole-grain barley and brown rice) and diets rich in non-digestible carbohydrates, respectively (De Filippo et al., 2010; Paturi et al., 2012; Liu et al., 2017).

Gut Microbiota and probiotics

Population of gut microbiota inside the gastrointestinal tract have modulated such that count of each genera are fluently consistent by having their own growth and every species play an important role in intestinal functioning. A balanced growth of bacterial species ingastrointestinal tract is desirable for optimum functioning of intestine. The growth of undesirable gut flora alters the balance and develops intestinal disorders subsequently. Probiotics benefit the host by stimulating the growth and activity of one or number of species in gut. The studies suggested that composition of bacterial communities in gastrointestinal tract was more stable during the period of probiotics treatment (Ki Cha *et al.* 2011). Recent technological revolution in DNA sequencing and developments in bioinformatics have provided advanced tools to explore research hypothesis linked with human microbiome and the mechanism behind treatment modalities that impact global composition and function of the microbial communities.

Cox et al., conducted a study on six month old infants using culture-independent method. Infants were treated daily with supplements of *Lactobacillus. rhamnosus* (LGG) and analysed their fecalmicriobiota. It was observed that there was an abundance of LGG and an increased index of evenness in fecal microbiota of infants, focusing ecological stability. Tannock et al., 2000 found that Lactobacillus rhamnosus and Enterococcus strains were retrieved during treatment at an increased number and frequency. These alterations could in turn promote or disrupt focal colonies of interacting microorganisms, especially in the context of colonization of multidrug resistant bacteria. The gut flora

is populated with the genus Lactobacillus and bifidobacterium and these strains are safe for human consumption and used to manufacture yoghurt and other fermented products. Consumption of probiotic improves diversity of gut flora and a healthy gut flora is a key to human health.

Probiotic and Human health

Table 1Studies related to impact of probiotic on diseases

S.No	Title	Conclusions	Reference
1.	Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children	Moderate quality evidence that probiotics are safe and effective for preventing C difficile associated diarrhoea	Goldenberg *et al*., 2017
2.	Probiotic supplementation for management of cardiovascular risk factors in adults with type II diabetes: A systematic review and metaanalysis	Probiotic consumption significantly decreased systolic blood pressure	Hendijani*et al.,* 2017
3.	Effects of probiotic supplementation in patients with type 2 diabetes: systematic review and meta-analysis	Probiotics significantly reduced fasting blood glucose compared with placebo	Akbari*et al.,* 2016
4.	Probiotics for preventing acute upper respiratory tract infections.	Probiotics were better than placebo in reducing the number of participants experiencing episodes of acute upper respiratory tract infections, the mean duration of an episode, antibiotic use, and related school absence.	Hao*et al.,* 2015
5.	Efficacy of probiotic use in acute rotavirus diarrhea in children: A systematic review and meta-analysis.	Probiotics exert positive effect on reducing the duration of acute rotavirus diarrhoea compared with control	Ahmadi*et al.,* 2015

Probiotics, Dysbiosis and Psychobiotics

The microbiota of the gut has both positive and negative effects on body. When the microbial community in gut eco system become imbalanced or when the pathogenic or opportunistic bacterial genera dominate over the good bacteria, it is known as“Dysbiosis”. Researches have confirmed that altered microbiota (Dysbiosis) plays an important role in causing several diseases and those diseases also have psychiatric component.(Sajadinejad*et al.,* 2012)Dinan et al., 2013 proposed the theory ofPsychobiotics. Psychobiotics are live microorganism which when consume in adequate amount benefits human with mental well beings. It is included an important class of probiotics species that has

huge advantage in psychiatric practices. Recent Studies on both human and animal model have shown that consumption of probiotic reduces the depression. (Table 2).

Table 2 Studies related to impact of probiotic in psychic disorders

Title of the study	Impact	References
Probiotic treatment (*Bifidobacteriumlongum subsp. longum 35624*™) affects stress responsivity in male rats after chronic corticosterone exposure	Positive	Has et al., 2020
Probiotic *Lactobacillus Plantarum 299v* decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo controlled study	Positive	Rudzki et al., 2019
Probiotic food consumption is associated with lower severity and prevalence of depression: A nationwide cross-sectional study	Positive	KimM.S et al., 2019
Probiotic *Bifidobacteriumlongum* NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome	Positive	Pinto-Sanchez et al., 2017
Effects of the probiotic *Bifidobacteriuminfantis* in the maternal separation model of depression	Positive	Desbonnet et al., 2010

Psychobiotics and Neurotransmitter

Psychobioticsare probiotic cultures that have the ability to produce neurotransmitters. Neurotransmitters are chemical compound that work as a messenger in body, transferring message between neurons, or from neurons to muscles. Neurotransmitters are not only produced in neural cell but are also synthesized in gastrointestinal system by gut microbiota. There is amassing confirmations that gut flora influence development of brain.. A study demonstrated that lactobacillus and bifidobacterium was isolated from human gut microbiota produced GABA when grown on glutamate containing media. (Barret*et al.*, 2012).Bacterial species that have been reported to produce particular neurotransmittersare as shown in Table 3.

Table 3 Name of Bacterial strain release neurotransmitters

Neurotransmitter	Bacterial strain	Reference
Dopamine	*Bacillus mycoides*	Tsavkelova*et al.*, 2000
Serotonin	*Lactobacillus plantarum*	Özog ˘ul*et al.*, 2012
GABA	*Lacobacillusbrevis*	Barrett *et al.*, 2012
Acetylcholine	*Lactobacillus plantarum*	Cho *et al.*, 2007
Histamine	*Lactobacillus plantarum*	Özog ˘ul*et al.*,2012

Neurotransmitters & mood swings

This is so far evidenced that imbalance of neurotransmitters in our brain impact and results different mood swings like depression, anxiety,irritability, insomnia, fatigue and so as behavioral addiction such as binge eating and gambling and when these chemicals are in healthy state generates a positive outlook towards things and clear imagination. Dopamine is a major neurotransmitter controls activity of brain. It is essential in treatment of important neurological (Parkinson's disease) and psychiatric disorders (schizophrenia, attention deficit hyperactivity disorders (ADHD). (Alexander *et al.,* 2004) Anxiety, depression and alzheimer's disease have been seen linked with GABA deficiency (Crayan and Kaupamann 2005). It is also found throughout endocrine nervous system and gastrointestinal tract. (Hyland and cryan., 2012).

Gutflora, Probiotics and Food additives

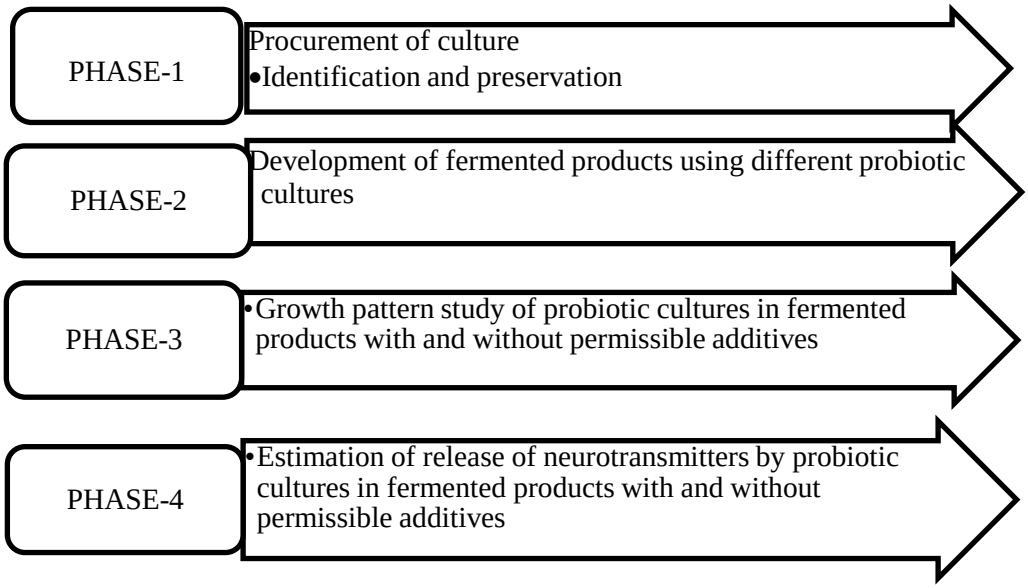
The market today is flooded with commercial foods which includes, processed, ready to eat and canned foods. These products for obvious reasons, contains significant amounts of food additives, added with the aim to increase shelf life and sensory characteristics of food. Food additives are the ingredients that are added to food to increase sensory quality of food such as colour, texture, appearance, taste etc. The usage of additive has increased dramatically since they were introduced for food preservation in the early 1800s. Currently, 2500 different permitted food additives are in use. The usage of food additives has increased to the extent that from the Food and Agriculture Organization (FAO) to every other nation across the globe has defined regulatory standards on their usage. The Food Safety & Standards Authority of India (FSSAI) too, has regulatory standards for usage of food additives. While discussing food additives, it is imperative to review the underlying implications of the use of additives on human health and gutflora.While on one hand consumption of probiotic foods are being prescribed to enhance the health and well-being of individuals by contributing the gutflora, on the other hand these probiotic foods are being laced with additives to boost the sensory characteristics, which in turn could do more damage than good. Probiotics foods products such as yoghurt, cheese, sour cream, butter milk and other fermented food products were introduced citing health benefits; seem to be increasing in demand in the market. Health benefits derived from these foods is through stabilizing the gut flora and through neurotransmitters released by the probiotic species,

neurotransmitters such as Dopamine and Serotonin which protect mental health by reducing symptoms of anxiety, stress and insomnia.

Evidently the question that arises would be on the probable effect of the food additives on the probiotic species.

Saccharin, a sweetener approved by WHO, which is added in rage of food and drinks was reported impact on gut flora of mice, it reduced Lactobacillus and Firmicutes and subsequently increased intestinal disturbance including proliferation of the Clostridiales and the Bacteroides.(Frankenfeld et al.,2015). Similarly, aspartame intake increased the number of *Clostridium leptum* and Enterobacteriaceae (Palmnas et al., 2014). Other sweeteners including Sucralose, Neotame and Cyclamate had also shown effect in perturbation of gut flora (Bian et al., 2017, Chi et al., 2018). Similarly, emulsifiers added in wide range of food products have been reported to show dysbiosis of gut flora(Viennois et al., 2017; Holder &Chassaing. 2018).

PLAN OF STUDY



5. METHODOLOGY

Procurement of culture

Cultures of *Bacillus spp* and *Bifidobacterium spp* will be procured from National Collection of Industrial Microorganisms (NCIM), Pune.

Identification and preservation of culture

Strain identity will be confirmed using culture, biochemical techniques as given in Rajashekhar *et al*., 2017 if molecular techniques will be required, 16sRNA molecular technique will be use for identification. Cultures will be preserved as Agar Slants and Glycerol Stock. They will be serialy sub-cultured from time to time.

Developing Probiotic fermented products

For preparation of fermented product (Yoghurt) using different species of probiotic bacteria, standard procedure will be followed.

Study on the effect of permissible additives on the growth of probiotic starter cultures

Standard Plate Count method will be used for estimating the culture concentration.Effect of food additives(Colours, Thickeners & Stabilizers) that are added in yoghurt in permissible limit on growth of probiotic cultures will be studied.

Estimation of neurotransmitter released by different probiotic bacteria in both type of fermented products without additives and fermented products containing additives

Neurotransmitters will be estimated in fermented product using High performance liquid chromatography (HPLC). (Tung et al., 2011)

STATISTICAL ANALYSIS

Relevant statistical test will be applied to evaluate the data acquiredthrough experiments.

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